LOGISTICS OF A PRACTICAL CLASS ON FACTORS AFFECTING DRUG METABOLISM AND DISPOSITION

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Desperate needs for health professionals is translated into yearly increases in university student admissions in many developing countries. Yet economic contraints dictate that subventions for teaching and research remain static or even decrease as student populations increase. Practical teaching, for example in Pharmacology, is very susceptible to inadequate funding because of capital and recurrent expenditure needed to procure and maintain laboratories, staff, animals, instruments and chemicals. Class demonstrations though providing a partial answer to the problem is beset with a number of disadvantages. Provision of qualitative training on quantitatively low funds demands ingenuity on the part of the lecturer in modifying and adapting standard teaching/learning processes while still achieving desired objectives. This paper illustrates such a procedure by describing the logistics of a practical class on "Factors affecting drug metabolism and disposition" to large undergraduate medical and/or pharmacy students, on limited laboratory space, staff and budget. A sample laboratory result is included.

FATE OF BENZO(A)PYRENE 7,8-DIHYDRODIOL-9,10-EPOXIDE (BPDE) IN INFANT SWISS WEBSTER MICE

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Infant Swiss Webster mice (15 day-old) were injected with 39 nmol [^{14}C]BPDE i.p., a dose known to induce lung tumors (Beuning et al., *PNAS*, 75, 5358, 1978). Conjugated metabolites in serum and in tissues were analyzed by thin layer chromatography. One hour after injection, 5% of the administered dose remained in plasma with over 90% of the radioactivity as conjugates (54% as glucuronides and 16% as glutathione conjugates). Presence of electrophilic metabolites in serum were determined indirectly by trapping with salmon sperm DNA and found to be less than 0.1% of injected dose (30 pmol/ml). Radioactivity in the intestines, representing mostly biliary metabolites, accounted for 10% of the dose. Glutathione conjugates predominated in the liver (2.8 nmol/g, 51%) and lung (2.8 nmol/g, 38%) whereas glucuronides were the major metabolites in kidney (5.7 nmol/g, 79%). Sulfate conjugates and tetrols constituted 10-40% of radiolabel in tissues. Radioactivity bound to DNA in liver, lung and kidney was 21.5, 42.6 and 7.77 pmol/mg, respectively. These results suggest that conjugates of BPDE, particularly those with glutathione, as well as BPDE may contribute to DNA binding in susceptible tissues. (Supported in part by NIH Grant CA 20807)

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STUDY OF RELATIONSHIP BETWEEN CONCENTRATION AND EFFECT OF BENIDIPINE HYDROCHLORIDE

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Benidipine hydrochloride,(±)-(R*)-3 [(R*)-1-benzyl-3-piperidyl]methyl 1,4-dihydro-2,6-dimethyl-4-(m-nitrophenyl) 3,5-pyridinedicarboxylate hydrochloride(KW-3049) is a novel, potent and long lasting calcium antagonist and is currently undergoing evaluation as a therapeutic agent against hypertention and angina. When benidipine was orally administered to essential hypertensive patients, anti-hypertensive effect was not directly related with its plasma concentration(1). After oral administration in spontaneously hypertensive rat, relationship between decrease of blood pressure and plasma concentration showed anti-clockwise hysterisis phenomenon. The effect is well described with a Hill-type function of drug concentration in the effect compartment. The calculated concentration in the effect compartment increased behind plasma concentration and decreased slowly. To investigate the propriety of effect compartment model, we measured plasma concentrations and concentrations in mesenteric arteries (model of target tissue) by GC-ECD after oral or intravenous administration in rats. When benidipine (1 mg/kg) was administered orally, Cmax values in plasma and mesenteric arteries were 1.44±0.98 ng/ml (mean ±S.D., n=8) and 4.7±3.5 ng/g tissue, respectively. MRT(plasma) and MRT (mesenteric arteries) were 3.6 and 9.7 hr, respectively. When benidipine (0.01 mg/kg) was administered intravenously, Cmax values in plasma and mesenteric arteries were 2.77±0.36 ng/ml (n=4) and 4.0±0.4 ng/g tissue. MRT(plasma) and MRT (arteries) were 2.1 and 7.5 hr, respectively. The concentrations in mesenteric arteries were higher and eliminated slower than plasma concentrations. This result may explain the pharmacodynamics of this drug.

Reference : 1.Noda,H.,et al.;Jpn.Pharmacol.Ther.,18,Suppl 4,21-29 (1990)

CARRIER-MEDIATED UPTAKE OF PRAVASTATIN BY RAT HEPATOCYTES IN PRIMARY CULTURE

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The transport mechanism of pravastatin, a new cholesterol-lowering drug, was compared in vitro with rat hepatocyte primary culture and mouse skin fibroblast(L-cells). The uptake of [^{14}C]-labeled pravastatin by cultured hepatocytes is temperature- and dose dependent. The temperature-dependent uptake as a function of [^{14}C]-pravastatin showed a saturation kinetics with Km=3.2x10^{-8}M and maximal uptake rate=62 pmol/mg protein/min. The uptake of pravastatin was inhibited significantly by metabolic inhibitors such as rotenon, oligomycin, actinomycin A and DNP. Unlabeled pravastatin as well as R-416 and R-195, structural analogs of pravastatin effectively competed the hepatic uptake of [^{14}C]-pravastatin at 37℃. These results indicated that pravastatin is taken up by the liver by an active transport. In contrast, the transport of pravastatin to L-cells was temperature-independent and non-saturable, suggesting that the uptake of pravastatin by L-cells is mediated by passive diffusion. A marked contrast in the uptake mechanism between hepatocytes and L-cells may account for a unique feature of this drug that the uptake and inhibition of cholesterol biosynthesis is selective to liver.

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THE EFFECTS OF COINDUCTION WITH TCPOBOP AND PB ON THE MONOOXYGENASE SYSTEM OF RATS

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1,4-bis[2-(3,5-dichloropyridyoxy)]benzene is Known as an inducer of the PB-type for mice but not for rats. TCPOBOP was administered to the Wistar rats preliminary treated with injection of phenobarbital (PB) (20 mg/kg). It has been found that in these case TCPOBOP prolongates the inducing effect of PB on the livers monooxygenase system. There was a marked increase in the biotransformation of pentoxyresorufine, testosterone and benzphetamine. The specific contents of cytochrome P-450IIB were also increased as determined by the ELISA method. The levels of mRNA were compared for the rats treated with PB only and PB-TCPOBOP using the specific oligonucleotides probes and cDNA for cytochrome P-450 (rats cytochrome P-450e). Similar results have also been obtained for the mice treated with isosafrole (IS) and 2,4,6-threephenyldioxane-1,3 (TFD). Earlier we have shown that TFD is an inducer of the PB type for rats but is inaffective for mice. The data obtained evidence that PB in rats and IS in mice induce expression of proteins which are involved into the mechanism of expression of cytochrome P-450IIB gene family.

CHEMICAL BASIS FOR R-(+)-PULEGONE MEDIATED TOXICITY

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R-(+)-pulegone (I), a monoterpene ketone, the major constituent of Mentha pulegium has been shown to be both hepatotoxic and pneumotoxic. Liver microsomal cytochrome P-450 is involved in the bioactivation of I to reactive metabolite(s) responsible for the toxicity.Menthofuran (II) has been identified as one of the metabolites of I and it is believed to be the proximate toxin derived from I. However, the mechanism of toxicity and the nature of the reactive metabolite(s) are not known.

In vivo studies revealed that 4-hydroxy-4-methyl-2-cyclohexenone (III), and p-cresol (IV) as part of the neutral metabolities derived from II. Incubation of PB-induced rat liver microsomes in the presence of NADPH and O_2 convert II to 2-Z-(2'-keto-4'-methyl cyclohexylidene) propanal (V,α,β-unsaturated-γ-ketoaldehyde). Besides, rat liver microsomes readily convert 4-methyl-2-cyclohexenone (VI) to III and IV in the presence of NADPH and O_2, further supporting the in vivo findings. These results suggest that microsomal cytochrome P-450 system converts II to its epoxide which can give rise to V. Further metabolism of V can yield p-cresol (IV) via the intermediacy of VI and III. It is resonable to assume that part of the toxicity mediated by I could be due to IV, a known liver and lung toxin. Besides V can also contribute to the toxicity by virtue of its ability to covalently interact with tissue macromolecules.

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CYTO-PROTECTIVE EFFECTS OF EBSELEN ON PARACETAMOL INDUCED HEPATOTOXICITY IN FRESHLY ISOLATED HEPATOCYTES FROM RATS

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The cytoprotective effect of ebselen, a new drug amongst others under development as an anti-inflammatory agent, on paracetamol induced cytotoxicity in rat hepatocytes was studied. Paracetamol was chosen as a model-toxin, since it is well known to be bioactivated in hepatocytes by ß-naphthoflavone inducible cytochrome P450's to N-acetyl-p-benzoquinone imine (NAPQI), a reactive metabolite upon overdosage with paracetamol causing intracellular protein- (PSH) and non-protein (GSH) thiol-depletion, lipid peroxidation and finally cell death. Our results show that ebselen can prevent paracetamol-induced hepatotoxicity in rat hepatocytes in a dose-dependent manner. At a concentration of 50 µM simultaneously added with paracetamol (1mM), ebselen prevented paracetamol-induced LDH leakage almost completely and prevented lipid peroxidation (LPO) and GSH-depletion partly. At higher concentrations, the protective effects of ebselen were even more complete. 50 µM of ebselen added to the hepatocytes one hour before paracetamol also inhibited LDH-leakage, LPO and GSH-depletion. When added 1 h after paracetamol, 50 µM of ebselen could not protect the hepatocytes against paracetamol toxicity anymore. Ebselen does not react with paracetamol directly. Moreover 50 µM of ebselen does not inhibit cytochrome P450 enzyme activity measured as ethoxycoumarine deethylation in freshly isolated hepatocytes. Possible mechanisms for the observed protective effects of ebselen are discussed.

N^+-GLUCURONIDATION OF ALIPHATIC TERTIARY AMINES, A GENERAL PHENOMENON IN THE METABOLISM OF H_1 ANTIHISTAMINES IN HUMANS.

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The H_1 antihistamine drugs have at least one aliphatic tertiary amine group in their structure. Quaternary ammonium-linked glucuronide metabolites have been identified in human urine for only two of these drugs. The availability of synthetic samples of such metabolites, prepared by a general method of chemical synthesis developed in these laboratories, facilitated study of whether their formation is a general phenomenon in the metabolism of H_1 antihistamines in humans. Nine representative drugs (chlorpheniramine maleate, cyclizine lactate, diphenhydramine hydrochloride, doxylamine succinate, pheniramine maleate, promethazine hydrochloride, pyrilamine maleate, terfenadine, tripelennamine hydrochloride) from each of the various structural classes of H_1 antihistamines were investigated. In each case small oral dose(s) were administered over no more than 6 h to two healthy volunteers and the total urine collected for 36 h. Metabolites were isolated by chromatography on a XAD-2 column followed by h.p.l.c. The structure of each metabolite identified as a N^+-glucuronide metabolite was confirmed by direct comparison of its FAB mass spectrum and chromatographic behaviour with that of a reference standard. In all but one case (terfenadine), metabolism by the N^+-glucuronidation pathway was observed in each of the volunteers. The mean percentage of dose excreted as the metabolite was greatest for cyclizine (14.3%), tripelennamine (6.5%) and diphenhydramine (4.0%). (Supported by MRC of Canada, PG-34.)

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PATTERNS OF ADENINE NUCLEOTIDES, LIPID METABOLISM AND CYTOCHROME P-450 IN THE LIVERS OF RATS TREATED WITH CORN OIL AND PHENOBARBITAL.

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Male Wistar rats were treated with corn oil (0.5 ml/100g) and/or PB (10 mg/100g). In the oil-treated rats the liver contents of acyl-CoAs, FFA, TG and PL increased significantly. The energy state of adenine nucleotides decreased. In the blood there was an increase in VLDL and decrease in HDL. PB alone did not influenced on the AN and acyl-CoAs in the liver, but FFA, PL were increased. There were no large changes in the blood lipids. In the rats treated with oil+PB the liver contents of acyl-CoAs, FFA and the energy state of AN were normal. The blood lipoproteins were also normal. The corresponding values of cytochrome P-450 were: Control - 0.91+0.15; Oil - 0.907+0.2; PB - 2,108+ 0.22; Oil+PB - 2.53+0.3 nmol/mg microsomal protein Under all conditions the cytochrome P-450/PL ratio remained constant. It is concluded that PB shifts the metabolism of FFA towads synthesis of PL, and normolizes the LP-spectrum in the blood under conditions of increased consumption of FFA. On the other hand, increased synthesis of membraneous phospholipids stimulates the inducing capability of PB by supplying membranes for incorporation of the cytochrome's apoprotein.

REACTION OF HEMOGLOBIN WITH FREE RADICALS: GLOBIN-HEME INTERACTION IN THE PROCESS OF HEMICHROME FORMATION.

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Hemoglobin (Hb) redox processes have been linked to toxic effects of drugs and to accelerating aging of red blood cells in several hematologic disorders. Redox intermediates produced by biological activation of drugs are free radicals. The reaction of drug free radicals with Hb destabilized the globin structure and Hb can be converted into hemichromes (Hc) that in vivo are the main constituents of Heinz bodies. We have investigated the role of carbon and oxygen-centered radicals in Hb oxidation and denaturation by using a model of radical attack on Hb in which radicals were externally generated (azocompounds) or were formed inside the Hb molecule through a redox reaction (phenazine). Azocompounds oxide Hb to metHb, Hc (partially reversible) and choleglobin while phenazine produces metHb and ferrylHb (completely reversible). Direct EPR and spin trapping techniques indicate that the major Hb damage is produced by carbon radicals of the azocompounds or by nitrogen radical of phenazine. The formation of irreversible Hc may be indicative of a role for the globin chain in the oxidative process. The electrophoretic analysis of Hb irreversibly modified by azocompounds showed a time-dependent loss of Hb monomer and the formation of Hb dimer and oligomers. We hypothesize that free radicals can interact with some aromatic aminoacids of the globin structure working as electron-transfer centers for the heme iron. In peroxidation reactions, Hb may exhibit in vivo radical trapping antioxidant behavior sensitive to the oxygen tension.

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THE USE OF SUBCELLULAR LIVER FRACTIONS AND ISOLATED HEPATOCYTES FOR THE STUDY OF SPECIES AND SEX DIFFERENCES IN DRUG METABOLISM. THE IN VITRO METABOLISM OF FLUNARIZINE IN RATS, DOGS AND HUMANS.

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The biotransformation of ^{3}H-flunarizine ((E)-1-[bis(4-fluorophenyl)methyl]-4-(3-phenyl-2-propenyl)piperazine dihydrochloride, FLUN) was studied in subcellular fractions and in hepatocytes of rats, dogs and man. The major metabolites were characterized by radio-HPLC co-chromatography and/or by mass spectrometric analysis.

In subcellular fractions of male rats, oxidative *N*-dealkylation at the 4-piperazine nitrogen resulting in bis(4-fluorophenyl)methanol, was the major metabolic pathway, whereas aromatic hydroxylation at the phenyl of the cinnamyl moiety resulting in hydroxy-FLUN, was the major *in vitro* metabolic pathway in subcellular fractions of female rats and dogs. In fresh cultured male rat and dog hepatocytes, aromatic hydroxylation was the major metabolic pathway whereas in 2- and 3-day old cultures, the contribution of the hydroxylation pathway to the metabolism of FLUN decreased and oxidative *N*-dealkylation became the major pathway.

In human subcellular fractions, aromatic hydroxylation was again the major metabolic pathway. In primary cell cultures of human hepatocytes, oxidative *N*-dealkylation at the 1- and 4-piperazine nitrogen and glucuronidation of bis(4-fluorophenyl)methanol were observed. The *in vitro* metabolism of FLUN in humans, resembled more that in female rats and in dogs than that in male rats.

Both the sex difference for the metabolism of FLUN in male and female rats and the species differences for the metabolism of FLUN between rat and dog described previously *in vivo*, were detected in the present *in vitro* study. It is concluded that *in vitro* metabolism studies with subcellular liver fractions provide valuable means for the study of species differences in the metabolism of xenobiotics, and that hepatocytes reflect rather well, both qualitatively and quantitatively, the hepatic metabolism in the intact species.

INHIBIRION AND INDUCTION OF CYTOCHROME P450 ISOZYMES IN MOUSE LIVER BY THE HERBICIDE SYNERGIST TRIDIPHANE

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The synergistic activity of tridiphane with triazine herbicides, such as atrazizne, is a result of its ability to inhibit glutathione conjugation of atrazine by plant glutathione transferases (a detoxication reaction). Very little is known, however, about the interactions of tridiphane with mammalian xenobiotic-metabolizing enzymes. Using microsomes from mice pretreated with phenobarbital and 3-methylcholanthrene, as well as purified P450 isozymes, tridiphane was shown to selectively inhibit P450IIB1, while activities catalyzed by P450IA1 were unaffected. More recently tridiphane has been examined for its ability to induce P450 in vivo. Male C57BL/6N mice were given tridiphane, 250 mg/kg ip for 3 days. Liver weight and P450 content were increased after tridiphane treatment, and SDS-PAGE showed an increase in microsomal proteins between 50-60K. No significant increases in enzymatic activities were observed with the substrates benzphetamine, p-nitroanisole, benzo(a)pyrene, or ethoxyresorufin. There was, however, a significant elevation in microsomal hydroxylation of lauric acid, an activity specifically associated with induction of P450IVA1. Western blot analysis using an antibody specific for P450IVA1 showed a dramatic increase in a microsomal protein of the same molecular weight as purified P450IVA1 thus validating induction of this enzyme.

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BIOTRANSFORMATION OF SCOPARONE IN PRIMARY HEPATOCYTE CULTURES DERIVED FROM RATS, HAMSTERS AND MONKEYS[1].

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The coumarine derivative scoparone is regioselectively demethylated yielding isoscopoletine and scopoletine. It has been reported that in rats the ratio of the formation rates of these two metabolites (isoscopoletin/ scopoletin; I/S ratio) is indicative for the contribution of several P450 enzymes to the biotransformation of scoparone. In vitro changes in the pattern of P450s may therefore be reflected in a shift of this ratio. Hepatocyte monolayers of rats, hamsters and monkeys lost P450, especially during the first 24 hours of cell culture, a loss which was paralleled by a decrease in total scoparone metabolism. In hamsters and monkeys, but not in rats, the scoparone metabolite pattern was altered during primary culture of liver cells. Possibly, in hamster and monkey liver cells the P450s involved in scoparone metabolism decrease at unequal rates during culture. In the rat, however, the rates of decay for all P450s involved seem to be more or less equal. Human hepatocytes did not secrete scopoletine in detectable amounts. The regioselective demethylation of scoparone is applicable to monitor changes in metabolic activities of P450 isoenzymes in vitro. The metabolism of scoparone in humans differs from that in the other mammals.

1 Biochemical Pharmacology; *in press*

EFFECTS OF CULTURE SYSTEM, MEDIUM SUPPLEMENTATION AND OXYGEN AVAILABILITY DURING PERFUSION ON THE GLUTATHIONE DEPENDENT DETOXICATION SYSTEM IN ADULT RAT HEPATOCYTES.

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In order to obtain more information concerning the effects of culture and medium conditions on the glutathione dependent detoxication system, reduced (GSH) and oxidized (GSSG) glutathione contents, glutathione reductase (GR) and glutathione peroxidase (GPx) activities were studied in both pure cultures of adult rat hepatocytes and their co-cultures with rat epithelial cells. The addition of 10 % fetal calf serum, 25 mM nicotinamide, 0.1 µM selenium and 2 % dimethylsulphoxide respectively to the culture medium was examined.

As far as the glutathione level is concerned, it was found that, after a pronounced initial increase, the intracellular GSH levels decreased in both culture systems. In pure culture no stable situation was obtained, whereas in co-culture levels comparable with those obtained in freshly isolated hepatocytes were quickly reached and maintained for at least 2 weeks. The intracellular GSSG levels were very low.

Supplementation of the culture medium had no effect on the GSH and GSSG contents of the hepatocytes in both culture systems.

In pure culture, as far as GR and GPx activities are concerned, after an initial increase, they decreased slowly and increased again at the end of the culture period. In co-culture too, there was an immediate increase in GR and GPx activities. After 2 days of culture, all activities decreased and reached low stabilized levels, which were maintained during the rest of the culture time. No effect was observed by the various supplements to the medium.

The hypothesis was made that the initial increases in GSH levels and GR and GPx activities and the increases at the end of the culture time in GR and GPx activities in pure culture, could be due to increased oxidative stress. The effects of oxygen derived from oxygen saturated perfusion solution is now under investigation and results will be shown. Anyhow, a conclusion which can already be made is that a pronounced difference exists between pure culture and co-culture as far as the glutathione dependent detoxication system is concerned : co-cultures reach more stabilized situations than pure cultures do. Therefore co-cultures provide a better model to study glutathione dependent detoxication.

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DETERMINATION OF STYRENE-7,8-OXIDE IN HUMAN BLOOD: A SENSITIVE BIOLOGICAL MONITORING OF STYRENE EXPOSURE?

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Exposure to styrene is controlled by ambient air monitoring. Biological monitoring is performed either by determination of inhaled styrene vapors in blood or end-exhaled air or by determination of the corresponding metabolites mandelic acid and phenylglyoxylic acid in urine samples.

Styrene is first oxidized by the microsomal cytochrome P-450 dependent monooxygenase system of the liver to styrene-7,8-oxide which is hydrolized by the epoxide hydrolase or conjugated by the GSH-transferase. Styrene-7,8-oxide is a mutagenic and carcinogenic intermediate of the biotransformation of styrene. The use of styrene-7,8-oxide should be discussed for biological monitoring in man. Kessler et al. have published a method for the determination of styrene-7,8-oxide in rat blood samples (Verhandlungen d. Deutschen Gesell. f. Arbeitsmedizin 29, 521, 1989).

Now we have developed an analytical procedure for the quantitative gas chromatographic determination of styrene-7,8-oxide in humans. Because of the GSH-transferase activity blood samples should be seperated immediately after sampling into cell fraction and plasma. 3 ml of the plasma were transfered to an extrelut solid phase, eluated with 10 ml purified pentane. The eluat was evaporated to 0.5 ml using nitrogen. Propylene oxide was used as internal standard.

For the gas chromatographic separation we used large volume on column injection technique. 50 µl were injected into the gas chromatograph equiped with 10 m desactivated fused silica retention gap connected with 60 m stabilwax column. Hydrogen was utilized as carrier gas with a flow rate of 40 cm/s.

The method was applied for the biological monitoring of 10 styrene exposed persons. The concentration of styrene-7,8-oxide was measured within a range of 1.1 µl/l - 3.9 µl/l plasma. The concentration is low, inter-individual differences in metabolism could cause the concentration range.

BEFIPERIDE: DISTRIBUTION IN RATS.

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Befiperide a product of DUPHAR research, has demonstrated in animal tests effects in several anti-psychotic models.

The following aspects of the fate of befiperide were studied:

1 The distribution of radioactivity in the rat after administration of [^{14}C]befiperide hydrochloride.
2 The binding of befiperide to melanin in the rat.
3 The binding of befiperide to human plasma

The results show:

1 After oral administration of radiolabelled befiperide the radioactivity in adrenals persists much longer than in other organs.

2 Concentration of radioactivity in eyes is even larger than in adrenals indicating a strong melanin binding.

3 Protein binding of befiperide to human plasma is more than 99%.

4 Human plasma displaces melanine from the melanin-befiperide complex.

Conclusion:

Befiperide is a compound with cumulation potential in the adrenals. It has a strong but reversible melanin binding, and binds to more than 99% to human plasma.

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METABOLIC ACTIVITIES OF CYTOCHROME P450IA SUBFAMILY ISOZYMES IN RODENT LIVER MICROSOMES AND NUCLEI

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Effects of 3-MC and 2,3,7,8-TCDD were compared on monooxygenase activities of nuclei and microsomes isolated from livers of Wistar rats and CBA mice. 3-MC- and TCDD-treatment exercised a stimulatory effect on benzpyrene-hydroxylase activity in both microsomal and nuclear fractions of of these rodent species. The increase of 3,4-benzpyrene biotransformation rate was 2-3-fold higher in nuclei of liver cells in rats treated with these xenobiotics compared with microsomal fractions. It should be noted that TCDD caused in mice a higher inducing effect on benzpyrene-hydroxylase activity in microsomal and nuclear preparations (500 and 900 %, correspondingly) than 3-MC(350 and 500 %) 3-MC and TCDD exercised a similar effect on 7-ethoxyresorufin-O-deethylation activities in mouse fractions (4.5 and 3.5 nmol of product/min mer mg protein in microsomes and 0.20 and 0.25 in nuclei, correspondingly). EROD activity in TCDD-microsomes of rat was 2-fold higher than in MC-microsomes(8.0 vs 3.6), while in nuclei those activities wererather similar (0.23 and 0.21). The rate of 7-methoxyresorufin O-demethylation was 1.5-fold higher in mouse TCDD-microsomes than in 3-MC-microsomes (4.1 and 2.8) The opposite picture was observed in rat microsomes (1.40 vs 1.72). In liver nuclear fractions of both species TCDD caused a higher stimulatory effect on this activity than 3-MC, that ovestepping being 5-fold in mice and 1.5 fold in rats.

Mab 1-7-1 AS A PROBE FOR ESTIMATION OF METABOLIC ACTIVITIES OF CYTOCHROMES P450IA IN NUCLEI AND MICROSOMES OF RODENT LIVER

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Western blot analysis with Mab 1-7-1 showed that 3-methylcholanthrene and tetrachlorodibenzo-*p*-dioxin induced *de novo* synthesis of both forms of cytochrome P450IA subfamily in microsomal and nuclear fractions of CBA mice and Wistar rats liver. The same Mabs were used for immunoinhibition of *in vitro* metabolism of the specific substrates for cytochromes P-450IA1 and IA2 3,4-benzpyrene, 7-ethoxyresorufin and 7-methoxyresorufin in rat and mouse microsomes and nuclei. It was shown that Mab 1-7-1 inhibited benzpyrene hydroxylase and 7-ethoxyresorufin-O-deethylase activity almost completely (70-80 %) in liver microsomes and nuclei of 3-MC- and TCDD-treated animals of both species. The same effect was shown on 7-methoxyresorufin-O-demethylase activity in microsomal and nuclear preparations of TCDD-treated and in microsomes of MC-treated animals. However, in nuclear preparations of MC-treated rats and mice it was shown only 10-20 % inhibition of 7-MeROD activity. These data could suggest that some other cytochrome P-450 isoform(s) that are not sensitive to Mab 1-7-1 participate in 7-methoxyresorufin metabolism in the nuclei of liver cells of MC-treated rodents. Detection of P450IA1 and P450IA2 quantities using Western blot data allows to estimate the contribution of each isoenzyme in xenobiotic biotransformation.

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SPECIES DIFFERENCES IN THE METABOLISM OF PROMEDROL, THE SULEPTANIC ACID 21-ESTER OF METHYLPREDNISOLONE.

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The metabolism of [^{14}C]-Promedrol, labelled in the carboxamide carbon of the suleptanate (N-methyltaurine[^{14}C]suberamide) side chain was examined in rats and dogs (5 mg kg^{-1} methylprednisolone equivalents, i.v.) and human volunteers (500 mg methyprednisolone equivalents per person, i.v.). In all three species 70-80% of the dose was excreted in the urine. A solid phase extract of the urines was subjected to HPLC with radiochemical and thermospray M.S. detection. It was shown that the major radioactive constituent in dog urine was suleptanic acid, the esterase cleavage product of Promedrol, whereas in man and rat the major constituent was the product of β-oxidation, N-methyltaurine succinamide. It is therefore the rat rather than dog which is a suitable model for extrapolation of toxicology to man.

MECHANISM OF PIPERIDINE METABOLISM *VIA* AN IMINIUM INTERMEDIATE

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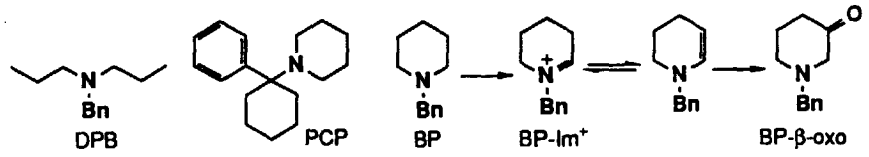
The piperidine ring is commonly involved in the structures of drugs and naturally occuring products, *etc.* The formations of chemically and pharmacologically active metabolites of cyclic tertiaryamines or their intermediates are of particular interest since such species may play an important role in, and may be often closely related to, the expression mechanisms of pharmacological and toxicological activities of mother compounds *in vivo*.

Oxidations of the piperidine ring by chemical model and liver microsomal systems were investigated with a simple piperidine derivative, *N*-benzylpiperidine (BP), as a substrate in order to probe the generality and the mechanism of the piperidine biotransformation. The piperidine ring of BP as well as that of phencyclidine (PCP) was suggested to be oxidized to a ketone at the β-position in *meso*-tetraphenylporphinatoiron(III) chloride (Fe(III)TPPCl)-oxidant system, and the reaction was expected to occur in liver microsomes-NADPH/O_2 system. The β-oxo formation was observed directly in the liver microsomal system, and found to be dependent on cytochrome P-450. The reaction was not observed when dipropylbenzylamine (DPB) was used as a substrate in the liver microsomal system.

We presumed a possible mechanism of β-oxo formation *via* BP-Im$^+$. BP-Im$^+$ and its free base, enamine, afforded large amounts of BP-β-oxo as well as BP-α-oxo in the chemical model and the microsomal systems. Therefore, the iminium species of piperidine ring can be a good precursor of further oxidized compounds including the β-oxo compound. In addition, the reactivities of *N*-benzylated 5-, 6-, and 7-membered cyclic amines , *i. e.* pyrrolidine, piperidine, and hexamethyleneimine, respectively, in the microsomal system were compared. β-Oxo formation was observed in the case of hexamethyleneimine as well as piperidine, but not the case of pyrrolidine. It is interesting that the β-oxo formation proceeds in 6- and 7-membered ring systems and does not proceed in 5-membered ring and acyclic ones.



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EFFECTS OF TETRAHYDROFURANE ON RAT LIVER MICROSOMAL MONOOXYGENASE ACTIVITIES

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Using of specific inhibitors and substrates allows to distinguish, identify and measure the individual isozymes of cytochrome P-450. We have been studied effects of tetrahydrofurane (THF) on rat liver microsomal metabolism of p-nitrophenol (p-NP), chlorozoxazone (CZ), and dimethylnitrosamine (DMNA). Treatment of rats with acetone and isoniazid resulted in increasing in p-NP, CZ and DMNA metabolism activities. CZ hydroxylase and DMNA demethylase activities were not changed in PB microsomes. p-NP hydroxylase activity in this microsomes was significantly increased. THF inhibited the metabolism of CZ in control, acetone and isoniazid microsomes. High-affinity DMNA demethylase was inhibited by THF only in a small degree. Less sensitivity of this activity to inhibition by THF may be due to different mechanism of this reaction . There was no effect of THF on p-NP hydroxylation in PB microsomes. It seems to exist other than P-450IIE1 isozymes involved in the metabolism of p-NP. THF administration to rats resulted in induction of P-450 total content and CZ hydroxylase activity, whereas p-NP was not changed. The inducing effect of THF is caused, probably, by its binding to P-450 and thus, protecting the enzyme from degradation.

EXPRESSION OF CYTOCHROME P-450 AND MONOOXYGENASE ACTIVITIES IN CULTURED FETAL RAT HEPATOCYTES

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Primary fetal hepatocytes in culture constitute an attractive "in vitro" model for the study of drug metabolism and toxicity and offer an approach to regulation problems concerning drug metabolizing enzymes.

Methods were developed to monitor monooxygenase activities on ongoing cultures directly in intact monolayers. Ethoxycoumarin deethylase, ethoxy- and pentoxy-resorufin dealkylase, benzopyrene hydroxylase, aldrin epoxidase and testosterone 16α,7α,2α,6ß hydroxylase activities on cells cultivated in the presence of several inducers were determined. These activities correlate with those found in the corresponding harvested and homogenized cells.

Fetal rat liver cells respond to the different inducers by expressing different P-450 isozymes, which in turn support the various monooxygenase activities: Testosterone is hydroxylated on various positions, P-450s from families IA1,IIB, IIIA are revealed by western blot.

Phenobarbital and benzanthracene both induce P-450IA1, EROD and AHH.

Dexamethasone (10^{-5}M) induces P-450IIIA and testosterone 6ß hydroxylase but does not modify EROD and AHH activities. At 10^{-6}M, dexamethasone allows the expression of various activities and their induction by PB: P-450IIB and IIIA, aldrin epoxidase, testosterone 2α,7α,6ß,16α hydroxylase.

Dimethylsulfoxyde (2%) induces AHH and EROD activities. DMSO also has a protective effect on the cellular membranes, maintaining existing enzymatic activities at a high level.

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THE DISPOSITION OF SALMETEROL IN ANIMALS AND MAN

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The disposition of salmeterol hydroxynaphthoate a new inhaled β2-adrenoceptor agonist with both bronchodilator and anti-inflammatory activity, was studied in laboratory animals and man.

[^{14}C]Salmeterol administered as its hydroxynaphthoate salt was rapidly absorbed across the gastrointestinal tract in animal species and man with peak levels observed within 2 hours. C_{max} was linearly related to the oral dose-level in mouse, rat and rabbit. In dog, the dose-normalised C_{max} was higher and reflected the greater oral bioavailability in this species. The plasma $t^1/_2\beta$, after intravenous administration, was 5 hours in rat and 2 hours in dog. The volume of distribution of salmeterol was significantly greater than total body water in both rat (40L/kg) and dog (6L/kg) and indicated high tissue uptake of the compound. Plasma clearance was high in rat (95ml/min/kg) and dog (30ml/min/kg).

Radioactive drug-related material was widely distributed throughout body tissues. The highest concentrations being present in bile, kidney, liver, g.i. tract, pituitary, lung, heart and bone marrow. Transfer of radioactive drug-related material across the placental barrier or into milk, was low.

In all species, the majority (55-75%) of the oral and intravenous dose was excreted in faeces. Biliary excretion, examined in rat and dog, accounted for 53% (0-27h) and 40% (0-8h) of the oral dose respectively, indicating good absorption across the g.i. tract. Enterohepatic circulation was significant in rat.

Salmeterol was cleared predominantly by metabolism in animals and man. The major routes of metabolism were species-dependent such that in mouse, rat and rabbit glucuronidation of parent compound was predominant; in dog phase I metabolism followed by sulphation, and in man aliphatic oxidation.

These studies indicate that in laboratory animals and man, salmeterol is well absorbed and extensively metabolised, with the metabolites being mainly eliminated by biliary excretion.

MODULATION OF THE CYTOTOXICITY OF ANETHOLE EPOXIDE BY THE CYTOSOLIC EPOXIDE HYDROLASE (cEH) INHIBITOR 4-FLUOROCHALCONE OXIDE (FCO) IN ISOLATED RAT HEPATOCYTES

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The 1,2-epoxide of the natural food flavour *trans*-anethole is a primary metabolite in the female rat and has a close structural similarity to the prototypical cEH substrate ß-methylstyrene oxide. We have examined the influence of the cEH inhibitor FCO, its inactive analogue chalcone oxide (1) and other modulators of epoxide metabolism on the cytotoxicity (assessed by LDH leakage and determination of intracellular GSH) of anethole in primary hepatocytes from female CD-SD rats.

While inhibition of microsomal epoxide hydrolase with trichloropropene oxide and cyclohexene oxide had no effect on anethole cytytoxicity, sustained depletion of GSH. acheived with diethyl maleate and buthionine sulfoximine together, resulted in a delayed (after 4h) and modest increase in the cytotoxicity of anethole. Only FCO gave a rapid and marked increase in cytotoxicity, with complete loss of cell viability within 2h. The lack of any effect of the non-inhibitor CO on anethole toxicity indicated that this was due to cEH inhibition.

It thus appears that cEH has an important rôle in detoxifying the epoxide intermediate arising from anethole and this is now being examined directly with a synthetic sample of the epoxide.

Supported by a grant from the FEMA Anethole Task Force. We thank B.D. Hammock for gifts of FCO and CO.

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METABOLIC FATE OF ^{14}C-FOY-305 IN MAN, RAT AND DOG

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N,N-Dimethylcarbamoylmethyl 4-(4-guanidinobenzoyloxy) phenylacetate methanesulphonate (camostat mesilate; FOY-305) is a synthetic proteolytic enzyme inhibitor indicated for use in the treatment of pancreatitis. Following 12-hour intravenous infusion doses of ^{14}C-FOY-305 to healthy male human subjects, >90% of the dose was excreted in urine (mostly within 24h post-administration), and no unchanged drug was detected in plasma (even during administration) due to its rapid rate of hydrolysis (*in vitro* $t_{\frac{1}{2}}$ <1 min.). The only two important metabolites of the drug in plasma and urine were identified as the primary products of its hydrolysis, *viz.* 4-(4-guanidinobenzoyloxy)phenylacetic acid (FOY-251) and 4-guanidinobenzoic acid (GBA). During the infusion period the former metabolite reached apparent steady-state concentrations, whereas the latter did not; both were characterised as highly-cleared compounds that undergo extensive tissue distribution. Systemic clearance of FOY-251 and GBA were 6.35 and 4.66 ml/min/kg respectively, and their apparent volumes of distribution (0.54 and 0.98 litres/kg respectively) approximated to the total boydwater volume. Protein binding of ^{14}C-FOY-305 in human plasma was low. Excretion profiles and metabolite patterns in rats and dogs after bolus intravenous doses of ^{14}C-FOY-305 were similar to those in man.

METABOLISM AND TOXICITY OF 3-METHYLTHIO PARACETAMOL IN MICE

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Paracetamol minor metabolite, 3-methylthio paracetamol (ASCH3) further metabolism and possible toxicity have been studied. ASCH3 was chemically synthesized (1). It was applied orally to mice in the dose of 1mM/kg. Mice were sacrificed and blood samples were collected 30min., 45min., 1.5h, 2h and 4h. Liver and kidney reduced glutathione concentrations were determined immediately. Serum concentrations of ASCH3 and its metabolites were measured by HPLC method (2). It was found that the major metablite in serum were ASCH3 glucuronide and paracetamol 3-methylsulphoxide (ASOCH3) but the concentration of ASCH3 were very low and not detectable 45min. after administration. It was found that liver GSH concentrations 4 hours after treatment were decreased for 24% but there were no significant changes of the kidney GSH concentrations. Paracetamol metabolite ASCH3 could be a contributing factor to paracetamol hepatotoxicity.

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FORMATION OF DNA-ADDUCTS OF VARIOUS AROMATIC HYDROXAMIC ACIDS AND HYDROXYLAMINES BY SULFOTRANSFERASE ACTIVITY IN RAT LIVER.

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Various aromatic hydroxamic acids and hydroxylamines are mutagenic/carcinogenic compounds that require metabolic activation in order to form reactive metabolites that may bind covalently to DNA and other cellular macromolecules.

We recently have shown that sulfotransferase activity is involved in the formation of various types of DNA-adducts of the biphenyl hydroxamic acids, N-hydroxy-4-acetylaminobiphenyl and its 4'-fluoro analogue, N-hydroxy-4'-fluoro-4-acetylaminobiphenyl through the formation of unstable, reactive sulfate esters (van de Poll et al., Carcinogenesis, 10, 2285-2291, 1989, and 11, 333-339, 1990). Formation of both N-acetylated and de-acetylated DNA-adducts was dependent on sulfotransferase activity. This contrasts with the formation of DNA-adducts of the structurally related hydroxamic acid, N-hydroxy-2-acetylaminofluorene, for which sulfotransferase activity is involved only in the formation of N-acetylated adducts in the liver of male rats.

Differences in hepatic sulfation capacity for the various hydroxamic acids and hydroxylamines that were used in this study, may explain these differences in the role of sulfation in DNA-adduct formation. Therefore, we have measured sulfation activity for these compounds in rat liver cytosol and in a partially purified sulfotransferase preparation. No differences in sulfation capacity were found for the various hydroxamic acids. Also, the hydroxylamines were sulfated at a similar rate by these two enzyme preparations. Therefore, other factors, like competition for detoxification pathways and/or rate of deacetylation of the various hydroxamic acids to their corresponding hydroxylamines, may determine the balance of formation of N-acetylated and de-acetylated DNA-adducts.

DETERMINATION OF THE STERIC AND ELECTRONIC FACTORS OF THE ENATIOSELECTIVE OXIDATION OF SULFIDES BY RABBIT LUNG FLAVIN CONTAINING MONOOXYGENASE

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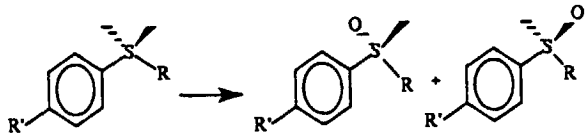
There are two major monooxygenase systems known to promote oxygenation of lipophilic exogenous substances prior to excretion, the the flavin containing monooxygenases (FMO, EC 1.14.13.8) and the cytochrome P450 monooxygenases. FMO consists of a family of isozymes which are organ and/or species specific and contains an oxygenated flavin adenine dinucleotide which is capable of transferring an oxygen atom to substrates containing nucleophilic S, Se, N, and P heteroatoms. We have carried out stereochemical studies on the oxygenation of a series of sulfides with rabbit lung and mini-pig microsomal and purified FMO, focussing our attention on two points: 1) the relationship of structure with enantioselectivity; and 2) the enantiointegrity of oxidation as a function of enzyme purification. The relationship of structure with enantioselectivity was probed with a series of prochiral sulfides. Rabbit lung FMO shows an apparent decrease in enantioselectivity for the (R) enantiomer with the size of R and no correlation with the electronic character of the R' group. The process of purifying rabbit lung FMO was shown to retain the enantioselectivity of oxidation by FMO, however a similar purification of mini-pig liver FMO proved to decrease the enantioselectivity. We conclude that the enantioselectivity of oxidation by rabbit lung FMO is dictated by steric, not electronic, factors and that the enantiointegrity of oxygenation is a good indicator of the quality of the FMO purification procedure



R= Me, Et, Pr, ^{i}Pr, ^{t}Bu, neo-Pentyl and R'= OMe, Me, H, CF_3, NO_2

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UDP-GLUCURONOSYLTRANSFERASE (UDPGT) ACTIVITY TOWARD DIGITOXIGENIN MONODIGITOXOSIDE (DT_1) IN HUMAN LIVER MICROSOMES

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Microsomal UDPGT exist in multiple enzyme forms which have different aglycone specificities. Recently, a distinct UDPGT ($UDPGT_7$ or DT_1-UDPGT) has been purified in rat. This enzyme glucuronidates DT1 (an intermediate metabolite of the cardiac glycoside digitoxin). Our aim was to further characterize the in vitro glucuronidation of DT_1 in human liver microsomes. The enzyme activity observed in non activated microsomes was very low (about 20 pmol/min/mg) as compared to previously published values in rat (104 pmol/min/mg) or mice (379 pmol/min/mg). DT_1-UDPGT activity was increased (180 to 220 % of control activity) by lubrol Px, Triton X100, or CHAPS. These results are in agreement with detergent effect observed by other authors in mice. The rate of DT_1 glucuronidation determined for 29 different human liver microsomes was highly variable (18 to 87 pmol/min/mg). The Km found was 4.5 µM. DT_1-UDPGT activity was tentatively correlated with other known UDPGT activities. No significant correlations were found between this activity and p-nitrophenol or 4-hydroxybiphenyl UDPGT activities. On the other hand a strong correlation ($r=0.64$; $^{*}p<0.05$) was observed between DT_1-UDPGT activity and digoxigenin monodigitoxoside (an other cardiac glycoside) glucuronidation rate. Finally, human DT_1-UDPGT exhibits specific properties as compared to animal DT_1-UDPGT previously characterized in rat and mice.

DEBRISOQUINE-TYPE OXIDATION METABOLIC PHENOTYPE IN DIABETIC PATIENTS

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The metabolic capacity of an individual may relate to susceptibility to certain diseases (such as cancer). Conversely, certain diseases are associated with alterations in drug elimination. Patients with diabetes have been reported to differ from nondiabetic individuals in their drug metabolizing capacity. The objective of this study was to determine whether debrisoquine-type oxidation capacity, which mediates metabolism of a large number of drugs, is different in diabetic patients than in the general population. To date, 12 diabetic patients and 47 healthy volunteers have been characterized as to their oxidation phenotype, using dextromethorphan (DXM) as the substrate. Subjects were given a single 30-mg oral dose of DXM, after which a quantitative 4-hour urine collection was obtained. Urinary molar concentrations of DXM and its hydroxylated metabolite dextrorphan (DX) were determined by HPLC. Subjects were classified as poor metabolizers (PM) of the debrisoquine type if the molar ratio of DXM to DX was less than 0.3. Subjects having a urinary molar ratio greater than 0.3 were considered to be extensive metabolizers (EM). Among the healthy subjects, 3 (6%) were identified as PM, which is consistent with the frequency distribution previously reported. All 12 diabetic subjects were characterized as EM. From our preliminary data, it appears unlikely that altered drug disposition in diabetic patients can be explained by an altered frequency distribution of the debrisoquine oxidative phenotype. The predominance of EM in our group of diabetics also suggests low likelihood of an etiologic factor for diabetes that is not rapidly detoxified by PM. (Supported in part by a Biomedical Research Support Grant through the UTHSCSA and NIH Grant M01-RR-01346)

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STRUCTURAL CHARACTERIZATION OF PANTOPRAZOLE URINARY, FECAL AND PLASMA METABOLITES AFTER SINGLE DOSE ORAL OR INTRAVENOUS ADMINISTRATION TO HUMAN VOLUNTEERS. G.Y. Kuo, T.J. Blake, K.M. Anderson, J. Kao, M. Carbonaro, C. Broom*, E. Sturm**, R. Huber**, B. Kohl** and D.M. Dulik, Dept. of Drug Metabolism, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406, USA, *Dept. of Clinical Pharmacology, SmithKline Beecham Pharmaceuticals, Welwyn, Herts., England, and ** Research Laboratories, Byk Gulden, Konstanz, FRG.

Pantoprazole (5-difluoromethoxy-2-[(3,4-dimethoxy-2-pyridyl)methylsulfinyl]-1H-benzimidazole) is an irreversible inhibitor of the parietal cell H^+/K^+-ATPase which is of potential value in the treatment of peptic ulceration and gastro-esophageal reflux disease. This compound is currently in Phase III clinical trials in Europe. A single intravenous (35 μCi, 60 mg) or oral (50 μCi, 80 mg) dose of ^{14}C-pantoprazole was administered to 3 healthy volunteers in sodium bicarbonate solution; urine and fecal samples were collected for 168 hr after dosing. Urine pH was observed to be >7. The majority of the total administered radiolabel (78-85%) was excreted into the urine within 48 hr. Approximately 10% of the dose was excreted into the feces within 72 hr. Quantification of urinary and fecal metabolites was done using reversed phase HPLC under neutral pH conditions followed by fraction collection and liquid scintillation counting. Seven metabolites (designated HU1-HU7) were detected in urine and the structures of five were assigned by a combination of thermospray LC/MS, FAB MS and proton NMR methods. Two fecal metabolites (HF7 and HF9) were also characterized by thermospray LC/MS. In a separate study, plasma metabolites of pantoprazole were isolated using HPLC with UV detection and characterized by mass spectrometry and proton NMR. Parent compound was the major circulating species; three additional metabolites were also observed. Pantoprazole is extensively metabolized after both i.v. and p.o. administration. The major pathway of biotransformation of pantoprazole in humans is p-O-desmethylation followed by sulfation or glucuronidation. Both reduction and oxidation of the sulfoxide moiety were also observed with and without subsequent conjugation. No metabolism specific to the route of administration (i.v. vs. p.o.) was observed.

ONE-ELECTRON REDUCTION OF QUINONES OF BHA AND OPP BY FLAVOENZYMES

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It has been reported that butylated hydroxyanisole (BHA), a food preservative, and o-phenylphenol (OPP), a fungicide for citrus fruits would be carcinogenic in rats. The oxidative metabolites are tert-butylhydroquinone (BHQ), tert-butylquinone (BQ) in BHA and phenyl-hydroquinone (OPPHQ), phenylquinone (OPPQ) in OPP. It has been reported that BQ and OPPQ have mutagenic activities. We have examined the mechanism of the enzymatic reduction of BQ and OPPQ in relation to the toxicity of BHA and OPP.

We have used some flavoenzyme systems: (1) NADPH cytochrome P450 reductase + NADPH (2) NADH cytochrome b_5 reductase + NADH (3) lipoamide dehydrogenase + NADH (4) xanthine oxidase + xanthine (X/XO system), using native, acetylated or succinoylated cytochrome c.

NADPH cytochrome P450 reductase catalyzed NADPH oxidation with the concomitant reduction of BQ or OPPQ to its hydroquinone (HQ). NADH cytochrome b_5 reductase catalyzed NADH oxidation with the concomitant reduction of BQ or OPPQ to HQ. In these conditions, the reduction of modified cytochrome c occurred. It was not inhibited by superoxide dismutase (SOD). These results suggest that BQ and OPPQ are reduced to their semiquinones (SQs) by these enzyme systems.

Lipoamide dehydrogenase catalyzed NADH oxidation with the concomitant reduction of BQ or OPPQ to HQ. In these conditions, the reduction of cytochrome c by this enzyme system occurred.

The reduction of cytochrome c by X/XO system in air was completely inhibited by SOD, but regained by adding BQ. X/XO system also reduced BQ to BHQ in N_2, in which conditions the reduction of cytochrome c occurred. The results indicate that X/XO system reduces BQ to a mixture of BHQ and semiquinone in air and in N_2.

It is concluded that these flavoenzymes mediate one-electron reduction of BQ and OPPQ to semiquinone radicals.

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TURNOVER OF NOVEL MODIFIED TISSUE PLASMINOGEN ACTIVATOR (E6010) IN RABBITS

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E6010 is a novel modified tissue plasminogen activator, whose plasma clearance is lower than that of tissue plasminogen activator (t-PA) in rats (1) and whose thrombolytic activity in plasma is also maintained longer than that of t-PA in rat arterio-venous shunt model (2). In the present study, the plasma level, tissue distribution and metabolism after intravenous administration of ^{125}I-E6010 (0.4 mg/kg) were examined and compared with those of ^{125}I-t-PA (0.4 mg/kg) in rabbits.

The plasma clearance (1.04 ml/min/kg) of E6010 was 10-fold lower than that of t-PA (10.53 ml/min/kg). The half-life of amidolytic activity was also prolonged as compared with that of t-PA. The radioactivity in plasma at 5min after dosing of ^{125}I-E6010 consisted of high molecular weight complexes (HMWCs) and free E6010 as well as t-PA. The ratio of HMWCs and free E6010 to the total radioactivity in plasma was 97%, which was higher than 64% in rats (1). At 1 hr after dosing of ^{125}I-t-PA, the radioactivity in plasma consisted of low molecular weight metabolites and/or free ^{125}I. On the contrary, at 1 hr after dosing of ^{125}I-E6010 the fractions of HMWCs and free E6010 still remained in plasma (81% to the total radioactivity in plasma). Tissue levels of radioactivity reached peak at 15min after dosing of ^{125}I-E6010. As for ^{125}I-t-PA, those reached peak at 5min. At 5min after dosing of ^{125}I-t-PA, liver level of radioactivity was the highest, followed by spleen, adrenal and plasma in descending order. On the other hand, at 5min after dosing of ^{125}I-E6010 plasma level was the highest, followed by liver, spleen, adrenal and kidney. The uptake of radioactivity into liver at 5min after dosing of ^{125}I-E6010 was 18.3% of the dose in contrast with ^{125}I-t-PA (70.1%).

These results suggest that the slower and less uptake of E6010 by liver resulted in the lower plasma clearance of E6010 than that of t-PA in rabbits, similar to the results in rats (1), and that E6010 may be more efficacious for the thrombolytic therapy than t-PA. (1) H. Mizuo et al., 49th International Congress of Pharmaceutical Sciences of F.I.P. '89 abstracts, p143 (1989)., (2) S. Suzuki et al., Thromb. Haemostas., 62, 543 (1989).

PURIFICATION AND CHARACTERISATION OF HEPATIC ALDEHYDE OXIDASE FROM GUINEA PIG.

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Aldehyde oxidase has become of interest as one of the xenobiotic - metabolising enzymes catalysing the oxidation of a variety of aldehydes and nitrogen-containing heterocycles , in the presence of O_2 and certain redox dyes , and the reduction of sulfoxides , N-oxides, nitrosamines, hydroxamic acids, azo dyes and nitropolycyclic aromatic hydrocarbons.

Guinea pig aldehyde oxidase was purified approximately 123-fold with a yield of 2% from liver cytosol using FFQ-Sepharose (FPLC) and Sephacryl S-300 HR column chromatography.
The final preparation was judged homogeneous by SDS-PAGE and existed as a dimer of sub-unit molecular weights of 130,000 and 145,000 daltons.
The purified enzyme showed similarities with the rabbit and guinea pig liver aldehyde oxidase reported by other workers with respect to its absolute absorption spectrum , molecular weight and catalytic activities.

Curently we are raising an antibody to the guinea pig enzyme in the sheep which will be used for its further characterisation.

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HALOPERIDOL INTERCONVERSION VARIABILITIES AND THE RH/HL RATIO IN SCHIZOPHRENIC PATIENTS

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Haloperidol (HL) is a potent dopamine antagonist used for the treatment of psychotic disorders. Metabolism of HL include N-dealkylation to inactive metabolites, and reduction to a pharmacologically less active metabolite, reduced haloperidol (RH). Interconversion of HL, in which RH is oxidized back to HL, has been observed in rats, guinea pigs, healthy volunteers, and patients. Animal studies indicated that the most likely site of interconversion is the liver. A single 0.5 mg/kg oral dose of HL and RH were administered to seven Chinese schizophrenic patients in a randomized crossover manner separated by 2 week interval. Blood samples were obtained for 72 hours after dosing and analyzed for HL and RH concentrations by HPLC/ECD. The clearance values for the interconversion process and other metabolic pathways of HL and RH were quantified using a pharmacokinetic interconversion model. The mean metabolic rate for oxidation of RH to HL (0.32 L/kg/hr) is less than that for reduction of HL to RH (0.35 L/kg/hr). However, there were up to ten fold differences in the oxidation and reduction capacities. There were also interindividual variabilities in the capacity of the N-dealkylation pathway (0.74 ± 0.36 L/kg/hr) and in the elimination of RH (0.37 ± 0.20 L/kg/hr). The RH/HL concentration ratio (0.40 ± 0.16) after 4 weeks of chronic dosing (10 mg twice daily) in these patients is related to variabilities in their individual metabolic capacities of the reduction/oxidation interconversion process and N-dealkylation pathway of HL. The reported interindividual and interethnic variabilities in RH/HL ratio, clinical response and adverse effects observed in patients treated with HL may be a reflection of genetic control of individual metabolic pathways associated with HL disposition.

BIOTRANSFORMATION OF SQ 32,756-^{14}C (BVaraU) IN SUBCELLULAR FRACTIONS OF LIVER FROM HUMANS AND RATS

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SQ 32,756 (BVaraU) is potent against herpes simplex virus type 1 and varicella zoster virus. SQ 32,756 is a prodrug that requires phosphorylation by virus-encoded thymidine kinase of infected cells to yield the triphosphate analog, which serves as an inhibitor of virus-encoded DNA polymerase. No phosphorylation of SQ 32,756 by uninfected host cells has been reported. The results obtained from *in vivo* metabolic studies indicated that after intravenous and oral administrations of SQ 32,756-^{14}C to rats, dogs, and humans, the major radioactive component in plasma, urine, and bile was unchanged SQ 32,756-^{14}C. A small amount of ^{14}C-(E)-5-(2-bromovinyl)uracil (BVU) was also detected. Since most nucleotides do not penetrate cell membrane and are usually confined intracellularly, concentrations of activated SQ 32,756-^{14}C (the mono-, di-, and triphosphate analogs) are not detectable in extracellular fluids. Thus, the metabolic activation of SQ 32,756-^{14}C cannot be studied using intact animals. To determine whether mammalian tissues are capable of phosphorylating SQ 32,756-^{14}C, the biotransformation of this compound was investigated in subcellular fractions of livers from humans and rats.

Incubation of SQ 32,756-^{14}C (2.9 and 29 μM) with the post-mitochondrial supernatant and cytosolic fractions of human and rat livers, fortified with an NADPH-generating system or NADH, resulted in no biotransformation of SQ 32,756-^{14}C. These results indicated that, under the experimental conditions of this study, SQ 32,756-^{14}C was stable against oxidative, reductive, and hydrolytic metabolic reactions in the livers of humans and rats.

The ATP-dependent phosphorylation of SQ 32,756-^{14}C to its monophosphate analog, BVaraUMP, was observed in the cytosolic fraction of livers that were obtained from humans and rats and had been kept frozen at -70° C. Little or no ^{14}C-BVaraUMP was detected in the incubations with the cytosolic fraction of fresh rat liver. Fresh human liver was not evaluated. No further phosphorylation of BVaraUMP to its di- or triphosphate analogs was observed in the cytosolic fraction of either human or rat liver. Some properties of this enzyme, presumably thymidine kinase, and the effects of pyrimidine nucleoside analogs on the phosphorylation of SQ 32,756-^{14}C in human liver will be discussed. The hydrolysis of ^{14}C-BVaraUMP to regenerate SQ 32,756-^{14}C, presumably catalyzed by 5'-nucleotidase, was also observed in the cytosolic fraction of human and rat livers. The formation of BVaraUMP may be regulated by the relative activities of thymidine kinase and 5'-nucleotidase in the hepatic cells.

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